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Chapter 2 - Patterns of gray matter loss in different manifestations of Alzheimer's disease

Chapter 2.1 Different patterns of gray matter atrophy in early and late-onset AD

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Abstract

We assessed patterns of grey matter atrophy according to age-at-onset in a large sample of 215 AD patients and 129 controls with voxel-based morphometry using 3-Tesla 3DT1 magnetic resonance imaging. Local grey matter amounts were compared between late- and early-onset AD patients and older and younger controls, taking into account the effect of APOE. Additionally, combined effects of age and diagnosis on volumes of hippocampus and precuneus were assessed. Compared to age-matched controls, late-onset AD patients exhibited atrophy of hippocampus, right temporal lobe and cerebellum, whereas early-onset AD patients showed GM atrophy in hippocampus, temporal lobes, precuneus, cingulate gyrus, and inferior frontal cortex. Direct comparisons between late- and early-onset AD revealed more pronounced atrophy of precuneus in early-onset AD patients and more severe atrophy in medial temporal lobe in late-onset AD patients. Age and diagnosis independently affected hippocampus; moreover, the interaction between age and diagnosis showed that precuneus atrophy was most prominent in early-onset AD. Our results suggest that patterns of atrophy may vary in the spectrum of AD.

Key words: Alzheimer's disease, early-onset, late-onset, MRI, voxel-based morphometry, grey matter atrophy, EOAD, LOAD

Introduction

The most salient characteristic of Alzheimer's disease (AD) on MRI is atrophy of the medial temporal lobe, including the hippocampus [1]. Heterogeneity in patterns of atrophy has been suggested however, and age of disease onset may be one of the factors related to the distribution of atrophy [2-4]. The few structural imaging studies published to date on this topic have shown that grey matter (GM) atrophy in early-onset AD seems to have a predilection for brain regions other than the medial temporal lobe, more located in the posterior and frontoparietal regions of the brain [5-11]. A former study by our group showed however that the hippocampus is similarly affected in younger and older AD patients when compared to age-matched controls [12]. The available literature on this topic lacks clarity due to small sample sizes resulting in lack of power and consequently insufficiently being able to adjust for multiple testing, the absence of direct comparisons between early- and late-onset AD, and the use of different image analysis techniques [5, 7, 9-11]. Some studies worked with a regions-of-interest approach, therefore missing patterns of atrophy in the rest of the brain [5]. Few studies used whole-brain approaches such as voxel-based morphometry (VBM) [13] which permits comparisons of GM volume at every voxel throughout the whole brain with no specific a priori hypothesis [5-7, 9]. An earlier study by our group addressed some of these problems, but still suffered from a small sample size and absence of an age-matched control group [6].

In addition to age at onset, APOE genotype has been suggested to exert regionally specific effects in the brain of AD patients [14-17]. Especially in early-onset patients, APOE genotype seems to modulate the disease [2]. In the present study we used VBM to detect patterns of GM atrophy in a large sample of early- and late-onset AD patients compared to age-matched controls, taking into account the potential modulating effect of APOE status.

Methods

Patients

We included 276 patients with probable AD and 140 patients with subjective complaints who visited the outpatient memory clinic of the Alzheimer Center of the VU University Medical Center (VUmc) between August 2008 and January 2011. All patients underwent a standardized one-day assessment including medical history, informant based history, physical and neurological examination, blood tests, neuropsychological assessment, electro-encephalography and magnetic resonance imaging (MRI) of the brain. Diagnoses of probable AD were made in a multidisciplinary consensus meeting according to the NIA-AA criteria [18]. The diagnosis of AD was confirmed by a presenilin 1 mutation in three patients and by autopsy in one patient.

As controls, we used patients who were labelled as having subjective complaints (normal clinical investigations, i.e. criteria for mild cognitive impairment (MCI) not fulfilled and no major psychiatric disorder). For inclusion in the present study patients had to fulfil the following inclusion criteria: 1) availability of a T1-weighted three-dimensional MRI scan (3DT1) at 3T GE MRI (details below), 2) age between 50-85 years, 3) availability of APOE genotype, and 4) availability of a Mini-mental state

examination (MMSE) score. Exclusion criteria were 1) poor MR image quality and/or large image artefacts, 2) failure of the image segmentation pipeline (details below), and 3) gross brain pathology other than atrophy, including severe white matter hyperintensities (WMH). Images of 215 AD patients and 129 controls were available for analysis. Both groups were categorised into a younger (< 65 years) and an older (≥ 65 years) group. This resulted in a study sample of 95 early-onset AD, 120 late-onset AD, 97 younger controls and 32 older controls. There were four patients who met the criteria of posterior cortical atrophy (PCA, 3 early-onset AD (3%), 1 late-onset AD (0.8%)) [19], three patients were diagnosed with logopenic variant primary progressive aphasia (lv-PPA, 1 early-onset AD (1%), 2 late-onset AD (1.6%)) [20]. The study was approved by the local medical ethics committee. All patients gave written informed consent for their clinical data to be used for research purposes.

APOE and cerebrospinal fluid

DNA was isolated from 10 ml blood samples in ethylenediaminetetraacetic acid (EDTA). APOE genotype was determined at the Neurological Laboratory of the Department of Clinical Chemistry of the VUmc with the LightCycler APOE mutation detection method (Roche Diagnostics GmbH, Mannheim, Germany). APOE data were available for all participants and were analyzed according to the presence or absence of an APOE ϵ_4 allele. APOE genotype was dichotomized in ϵ_4 carriers versus non-carriers. Cerebrospinal fluid (CSF) was obtained by lumbar puncture. Amyloid- β_{1-42} ($A\beta_{42}$), total tau, and tau phosphorylated at threonine-181 (Ptau-181) were measured by sandwich ELISA (Innogenetics, Gent, Belgium) [21]. CSF analyses were performed at the VUmc Department of Clinical Chemistry. Cut-off levels in our lab are as follows: $A\beta_{42} < 550$, total tau > 375 , and ptau > 52 [21]. CSF was available for 268 subjects (early onset: $n=81$; late onset: $n=92$; young controls: $n=72$; old controls: $n=23$).

MR image acquisition and review

Imaging was carried out on a 3.0 Tesla scanner (SignaHDxt, GE Healthcare, London, United Kingdom) using a 8-channel head coil with foam padding to restrict head motion. The scan protocol includes a whole-brain 3DT1 fast spoiled gradient echo sequence (FSPGR; TR 708 ms, TE 7 ms, flip angle 12° , 180 sagittal slices, field of view 250 mm, slice thickness 1 mm, voxel size $0.98 \times 0.98 \times 1$ mm) which was used for VBM. In addition, our standard MRI protocol includes 3D Fluid Attenuated Inversion Recovery (FLAIR), Dual Echo (PDT2), and Susceptibility Weighted Imaging (SWI). All scans were reviewed for brain pathology other than atrophy by an experienced radiologist. WMH were rated with the Fazekas scale [22], a 4-point rating scale, which provides an overall impression of the presence of WMH. Subjects with Fazekas scale score of 3 were excluded. Atrophy of the medial temporal lobe (MTA) was rated using a 5-point visual rating scale (0=absent – 4=severe) based on the height of the hippocampal formation and the width of the choroid fissure and the temporal horn [23]. Posterior atrophy (PA) was rated using a 4-point visual rating scale (0=absent – 3=end stage atrophy) based on the posterior cingulate- and parieto-occipital sulcus and sulci of the parietal lobes and precuneus [23, 24].

Voxel-based morphometry

DICOM images of the FSPGR sequence were corrected for gradient nonlinearity distortions and converted to Nifti format. The linear transformation matrix to MNI space was calculated using FSL-FLIRT [25] and used to place the image coordinate origin (0,0,0) on the anterior commissure by using the Nifti s-form.

The structural 3DT1 images were then analyzed using a modified voxel-based morphometry pipeline in Statistical Parametric Mapping (SPM8; Functional Imaging Laboratory, University College London, London, UK) implemented in MATLAB 7.12 (MathWorks, Natick, MA). In the first step VBM automatically identified GM, white matter (WM) and cerebrospinal fluid (CSF) of all scans. Based on these segmentations the volumes (l) of GM, WM and CSF voxels were determined separately for each scan and summed up to calculate total intracranial volume (TIV). After this segmentation process the images were rigidly aligned. Next, a DARTEL template of the grey matter of all scans was created by nonlinearly aligning the GM images to a common space [26]. The native GM and WM segmentations were spatially normalized to the "DARTEL" template by applying the individual flow fields of all scans, using modulation to compensate for volume changes due to compression and/or expansion. Images were smoothed using a 4 mm full width at half maximum (FWHM) isotropic Gaussian kernel. Images were visually inspected at every processing step.

Voxelwise statistical comparisons between groups were made to localize GM differences by means of a full factorial design which automatically models interactions between the factors. We used diagnosis (AD vs controls) and age (<65 vs ≥65 years) as factors with independent levels with unequal variance, using absolute threshold masking with a threshold of 0.2 and implicit masking. Sex and TIV were entered as covariates. To avoid the arbitrary dichotomization of age, we additionally used an ANCOVA model with diagnosis (AD vs controls) as factor with independent levels with unequal variance, using absolute threshold masking with a threshold of 0.2 and implicit masking. Age, sex and TIV were entered as covariates. An interaction with age (continuous measure) and diagnosis was modelled.

To test group differences *post hoc*, we used separate two-sample t-tests to compare early-onset AD with young controls, and late-onset AD with old controls, adjusted for sex, age, and TIV. Additionally, we directly compared early-onset AD with late-onset AD and young controls with old controls. In these comparisons, sex, MMSE and TIV were adjusted for. In a second model, we additionally adjusted for APOE genotype.

Finally, we assessed correlations between GM atrophy and MMSE by using MMSE score as covariate in the "one-sample t-test" set-up in SPM for the early- and late-onset AD group separately. The threshold for statistical significance in all VBM analyses was set to $p < 0.05$ with family wise error correction (FWE) at the voxel level and an extent threshold of 0 voxels. For visual representation we used an uncorrected threshold of $p < 0.001$.

Volumetry of hippocampus and precuneus

To visualize the associations between diagnosis and age on the one hand and changes in gray matter volume on the other hand, we extracted the volumes of the hippocampus and the precuneus using the Individual Brain Atlases as implemented in the Statistical Parametric Mapping (IBASPM) toolbox [27], a fully automated method

(<http://www.thomaskoenig.ch/Lester/ibaspm.htm>) based on the SPM software package (<http://www.fil.ion.ucl.ac.uk/spm>) in the following manner: Each normalized individual voxel of the native segmented GM density maps from the VBM pipeline was anatomically labelled, based on an Automatic Anatomic Labelling (AAL) atlas of predefined structures and taking into account the transformation matrix obtained in the normalization process [28]. An individual brain atlas that consisted of 84 different GM areas was created for each participant.

The volume (cm³) of each identified structure was calculated with the IBASPM volume statistic function. Volumes for the left and right hippocampus and left and right precuneus were summed and transferred to SPSS. Segmentation results were visually inspected for accuracy, and none was discarded.

Statistical analysis

Statistical analyses of clinical data were performed by means of SPSS for Windows version 15.0 (SPSS Inc., Chicago, Ill., USA). We used Student's t-tests, Mann-Whitney U-tests and Pearson Chi-Square tests to compare groups where appropriate. To estimate the combined associations between age and diagnosis (independent variables) and the volumes of hippocampus and precuneus (dependent variables) we used linear regression analyses. If there was no significant interaction between age and diagnosis, it was left out of the model. Sex and TIV were used as covariates. In a second model, we added APOE genotype as an additional covariate. The level of significance was set at $p < 0.05$.

Results

Demographic data for all patients ($n=215$) and controls ($n=129$) are summarized in table 1. Late-onset AD patients had a lower education and were more often APOE carrier than older controls. Early-onset AD patients were older and were more often APOE carrier than younger controls. Both AD groups differed from the control groups in all CSF biomarkers. Early-onset AD patients did not differ from late-onset AD patients regarding APOE status or CSF biomarkers. Younger controls had a smaller TIV than early-onset AD patients. Late-onset AD patients had higher MTA scores than early-onset AD patients and than old controls. Old controls had higher MTA scores than the young controls and early-onset AD patients had higher MTA scores than young controls. PA scores differed between old and young controls, between late-onset AD patients and old controls and between early-onset AD patients and young controls with the first groups having the higher scores. Late-onset AD patients did not have higher PA scores than early-onset AD patients. Late-onset AD patients performed worse on the delayed recall task of the Dutch version of the Rey auditory verbal learning task (RAVLT) than early-onset AD patients but there was no difference in performance on the total immediate recall. Late-onset AD patients performed better at the Trail making test A and B (TMT A, B) than early-onset AD.

The full factorial design showed main effects of diagnosis and age on patterns of GM reduction. A diagnosis of AD was associated with reduction of GM throughout the brain, especially in medial temporal lobe, hippocampus, and cingulate gyrus. Older

age was associated with GM reduction in medial temporal lobe and cerebellum. Furthermore, there was a significant interaction between age and diagnosis ($p < 0.05$ FWE), implicating that early onset AD patients had more pronounced GM reduction in the precuneus than in late onset AD patients.

An additional ANCOVA modelling age as a continuous factor confirmed main effects of diagnosis and age on patterns of GM reduction. A diagnosis of AD was associated with reduction of GM throughout the brain, especially in medial temporal lobe, hippocampus, and cingulate gyrus. Furthermore, there was a significant interaction between age and diagnosis ($p < 0.05$ FWE), showing that with an earlier onset of AD, there was more pronounced GM reduction in the precuneus and right temporal gyrus whereas the later the onset of AD, the more pronounced atrophy in medial temporal lobe and cerebellum occurred (figure 1).

For *post hoc* comparisons we stratified the groups based on age and used independent t-tests which showed that compared with older controls, late-onset AD patients showed GM reductions in hippocampus, left parahippocampal gyrus, right temporal lobe (middle temporal gyrus, superior temporal gyrus), left (inferior) parietal lobe and cerebellum ($p < 0.05$ FWE; figure 2). The reverse contrast showed no GM reductions in old controls compared to late-onset AD patients. Compared to younger controls, early-onset AD patients showed widespread reductions in GM in hippocampus, parahippocampal gyri, temporal lobes (middle and inferior gyrus), parietal lobes (primarily precuneus and angular gyrus), posterior and anterior cingulate gyrus, and inferior frontal cortex ($p < 0.05$ FWE; figure 2). The reverse contrast showed no GM reductions. In a direct comparison between early-onset and late-onset AD we found that late-onset AD patients had less GM in hippocampus and parahippocampal gyri, and cerebellum. By contrast, early-onset AD patients had less GM in the right precuneus despite their younger age ($p < 0.05$ FWE). When we compared both control groups, older controls had less GM in hippocampus, parahippocampal gyrus and middle temporal gyrus ($p < 0.05$ FWE). The reverse contrast showed no GM reductions in younger controls compared to older controls.

Additional adjustment for APOE did not change the results of any of these comparisons essentially, nor were there any interactions between age and APOE.

In an additional VBM analysis, we studied the correlation between regional GM reductions and dementia severity as measured by MMSE in AD patients. Lower MMSE scores were correlated with less GM in left temporal lobe ($p < 0.05$ FWE; figure 4). Separate correlation analyses in the late- and early-onset AD groups revealed comparable patterns in both groups.

Subsequently, we performed linear regression analyses to assess the combined effects of age and diagnosis on the extracted volumes of precuneus and the hippocampus. Volumes of the hippocampus were independently affected by diagnosis ($\beta(\text{SE}) = -1.944(0.135)$, $p < 0.001$) and age ($\beta(\text{SE}) = -0.068(0.008)$, $p < 0.001$). Volumes of the precuneus were also predicted by both diagnosis ($\beta(\text{SE}) = -14.967(2.764)$, $p < 0.001$) and age ($\beta(\text{SE}) = -0.125(0.036)$, $p = 0.001$). There was an interaction between diagnosis

and age ($p < 0.001$; figure 5), showing that precuneus atrophy is most prominent in early-onset AD. Additional adjustment for APOE did not change these results essentially, nor were there any interactions between age and APOE.

Discussion

The main findings of this study are that compared to younger controls, early-onset AD patients showed widespread GM atrophy throughout the brain (medial temporal lobe, precuneus, cingulate gyrus, frontal lobe) whereas late-onset AD patients showed a more specific pattern of GM atrophy, predominantly restricted to the medial temporal lobe and cerebellum. Direct comparisons revealed more pronounced GM atrophy in the precuneus of early-onset AD patients despite their younger age.

The generalizability of the few former studies on this topic is hampered by their small sample sizes [7-10]. In the current study we were able to show widespread GM atrophy in posterior and frontoparietal cortex in early-onset AD in a large sample. In this way, we replicated an earlier, preliminary finding from our group in a completely independent sample and found that age-at-onset modulates the distribution of GM involvement [6]. In late-onset AD, we observed a more specific pattern of GM atrophy in the medial temporal lobe and cerebellum. The linear regression analyses showed that hippocampal volume is independently affected by both age and AD, resulting in especially severely affected hippocampi in late-onset AD. Nonetheless, hippocampi of early-onset patients were also smaller than those of controls of their own age, confirming earlier results from our group and others [12, 29].

There are a number of possible explanations that may underlie our finding of more widespread atrophy in early-onset AD. First, knowing that neurofibrillary tangles originate in the medial temporal lobes, later spreading throughout the cortex, a possible explanation of the more widespread atrophy in early-onset AD would be that they were in a more advanced disease stage. Disease severity as measured with MMSE was similar for both age groups however, rendering this an unlikely explanation.

Second, early-onset AD patients may have more cognitive reserve, explaining the finding of more widespread atrophy in the presence of similar performance on MMSE [30-32]. If this were true, one would expect that when the burden of neurodegeneration reaches a certain level, clinical performance drops at a faster level, as has also been described for patients with a high education [33-35]. This would fit with the observation that early-onset AD patients show more rapid cognitive decline [36-38].

Third, the different patterns of GM atrophy may reflect a difference in regional vulnerability to the disease. Findings of previous reports provide evidence that the origin and spread of tau pathology originating from the transentorhinal and hippocampal area might not be the only pattern of pathological progression in AD. Spreading of AD pathology might differ between individuals and certain subtypes of AD may have proportionally greater involvement of the cortex than of the hippocampus. Age of onset could be a driving factor in this regional vulnerability as

other studies showed that AD patients with a hippocampal sparing subtype were younger at age-at-onset, had a shorter disease duration, and more widespread cortical involvement than the typical and limbic-predominant AD subtypes [4, 39]. This might imply that the Braak stages as described in the early nineties do not hold for all AD patients but need to be adapted for specific subgroups [40, 41]. Differences in clinical profile provide further support for this notion [42-45]. The cognitive profile in early-onset AD often includes prominent non-memory problems such as apraxia, aphasia, and visuospatial dysfunction, seemingly befitting our finding of widespread patterns of GM atrophy in the frontoparietal and posterior cortices. The clinical profile of late-onset AD is typically characterized by memory impairment, in line with their more specific pattern of GM atrophy in the medial temporal lobe. Atypical, focal, clinical syndromes like the logopenic variant of primary progressive aphasia (LPA) or posterior cortical atrophy (PCA), which could be misdiagnosed as early-onset AD, could be another explanation as these groups share largely overlapping patterns of atrophy with early-onset AD patients [46-49]. However, these cases are rare in our sample, not restricted to the early onset AD group and all are underpinned by AD pathology [50-53] rendering it unlikely to be the only cause of determined differences between early- and late-onset AD.

Differences in regional vulnerability are probably explained by genetic and/or biological factors that predispose for both an earlier age-at-onset and macroscopic changes in the brain. APOE is a genetic risk factor for AD which has been associated in nondemented populations with more severe brain atrophy of regions typically hit by the disease, such as the medial temporal lobe [14, 15, 17]. It has been postulated that APOE genotype has a modulating effect on the relationship between age at onset and regional GM vulnerability [2]. In the current study, we also investigated this hypothesis. Contrary to our expectation, we found no such effect of APOE. This result is in line with other studies on this topic which also did not find an effect of APOE in combination with age-at-onset [7, 9, 54]. There may be a number of explanations for our negative finding. First, there may have been insufficient statistical power to show an effect of APOE. However, the large sample size of this study renders this explanation unlikely. Second, APOE may act at another stage of the cascade of events leading to clinical AD. In former studies of our group, we have shown effects of APOE genotype on brain activity and cognitive functioning, suggesting that APOE influences brain networks instead of cortical regions [36, 54, 55]. If this is true, then there must be other (genetic) factors that modulate patterns of atrophy. Autosomal dominant mutations could be a driving factor but are rare and their impact on patterns of atrophy has been poorly understood so far [56-58]. The effect of newly identified mutations with small effects on pattern of atrophy and age-at-onset remains to be elucidated [59, 60].

Our finding of more severe cerebellar atrophy in late onset AD patients was rather unexpected, but in line with other studies on dementia and MCI [51-63]. There are several potential explanations for this finding. First, it could be an age effect. It has been shown that in cognitively normal individuals there is GM atrophy in primary motor, sensory, and heteromodal association areas, as well as the cerebellum with

chronological age [29, 64, 65]. Atrophy with aging in these areas, particularly in the cerebellum, may reflect altered plasticity in efferent and afferent pathways that are involved in visual, sensory [66] and motor/mobility [67] functions in the elderly. Second, it could be speculated that there is a joint effect of age and AD as we only found cerebellum atrophy with age within the AD group (not between old and young controls). Third, Glodzik et al. found that subjects with hypertension showed significantly less GM in the cerebellum than subjects without hypertension [68]. As hypertension is a risk factor for AD and more common with older age, it is possible that hypertension in the older participants is one reason of the grey matter loss in the cerebellum as it has been found that cerebellar Purkinje cells are particularly susceptible to ischemia [69]. Elaborating on this, it has been shown that AD-type brain pathology, along with hypertension, is associated with white matter changes seen on MRI [70]. As signal differences in the cerebellum are often observed with SPM due to the registration difficulties in this region it could be possible that the results in the cerebellum reflect changes in the white matter rather than in the grey matter.

The results of the linear regression analyses clearly show the different relationships of hippocampus and precuneus with age and diagnosis. In contrast to medial temporal lobe atrophy, which increases with ageing and additionally increases with disease, precuneus atrophy is most outspoken in early-onset AD and therefore we find a flat slope with age in AD patients, whereas in controls a steep slope describes the decline of the precuneus with increasing age. The scores of the parietal rating scale show the same pattern. The reason why precuneus atrophy does not seem to increase with age in AD patients is not clear. Possibly, it reflects a very AD-specific phenomenon: independent of age-at-onset, precuneus volume will reach a bottom level. This would be comparable to the pattern which is also seen in AD-specific markers such as amyloid-beta concentration in CSF or amyloid-PET [71-74]. Another potential explanation of the flat slope in AD patients could be that precuneus atrophy is relatively more pronounced in early-onset AD as the difference with their own controls is larger than in late-onset AD patients.

One of the strengths of this study constituted our large sample size, due to which we had enough power to detect even subtle differences in GM atrophy in a direct comparison between early-onset AD and late-onset AD patients using FWE-correction to adjust for multiple testing. Another strength was the carefully applied VBM pipeline including visual checks of the results of each step. A possible limitation of this study is that we did not have post-mortem data available, so the possibility of misdiagnosis cannot be excluded. Nevertheless, we have an extensive standardized work-up and all patients fulfilled clinical criteria of probable AD. Furthermore, CSF biomarkers were available for the majority of patients and average biomarker levels were congruent with a diagnosis of AD in both groups, rendering the possibility of misdiagnosis less likely. Another limitation could be the fact that we used persons with subjective memory complaints as control group, as these subjects are known to have an increased risk of progression to dementia. The main question in this study however involved a comparison between early- and late-onset AD, which is not influenced by the control group. The use of IBASPM to select precuneus and hippocampus is not

totally independent from our VBM results as it uses the segmentations of the VBM pipeline. Nevertheless, IBASPM relies on the AAL atlas and selects bigger regions than only the significant clusters from the VBM analyses. Therefore, it adds extra support to our VBM results. For the VBM analyses we used masking with an absolute threshold of 0.2 which is considered as a relative high threshold. Because of this strict threshold, it is possible that voxels were excluded from the analysis where the brains are most vulnerable to atrophy [75] and results could be narrowed. However, the location of significant different voxels met our expectations and that of other studies. Furthermore, we repeated the full factorial analysis with an absolute threshold of 0.1. The results did not essentially change compared to those with a threshold of 0.2 and therefore support our results even with a stricter threshold. The same is applicable to the size of our smoothing kernel. Whereas a lot of studies use larger smoothing kernels (SPM8 reports an estimated smoothness of FWHM 8-9 mm) in our study a kernel of 4 mm was chosen because the increased accuracy of the DARTEL registration algorithm means that smaller kernels should be sufficient to correct for misalignment. As the kernel size increases, so does the extent of the findings [76], we believe that the small kernel size underlines the validity of our results.

The findings of the current study have important implications. The diagnosis of early-onset AD is often only made after years of delay. Early-onset patients often have a different clinical presentation, and at first sight, their scan may appear relatively normal. All too often, posterior and frontoparietal atrophy are overlooked in the daily clinical practice of memory clinics, explaining why the disease is often not appropriately diagnosed. To overcome this problem, it is important to compare patients with a reference of their own age category. Moreover, in younger patients, the posterior part of the brain – especially the precuneus – may provide the most valuable information.

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Table and Figures

Table 1. Demographic characteristics

	Alzheimer's disease		Controls	
	Late-onset	Early-onset	Old	Young
N	120	95	32	97
Age, years	72 ± 5 ^{##}	60 ± 4	71 ± 4 ^{##}	58 ± 4 [#]
Sex, female	55 (46%)	52 (55%)	16 (50%)	46 (47%)
Level of education*	5 ± 1	5 ± 1	6 ± 2 [#]	5 ± 1
MMSE	21 ± 5	20 ± 6	28 ± 2 [#]	28 ± 2 [#]
RAVLT [§] total immediate recall	21 ± 7.6	23 ± 8.3	40 ± 7.6 [#]	43 ± 8.8 [#]
RAVLT [§] delayed recall	1.4 ± 2 ^{##}	2.4 ± 2.5	9 ± 2.8 [#]	9 ± 2.7 [#]
TMT A [§]	81 ± 51.8 ^{##}	113 ± 79.2	43 ± 14.8 [#]	42 ± 39.4 [#]
TMT B [§]	261 ± 161.1 ^{##}	330 ± 219.3	114 ± 55.1 ^{##, #}	98 ± 117 [#]
APOE genotype, ε4 carriers	80 (66%)	61 (64%)	7 (22%) [#]	37 (38%) [#]
Aβ ₄₂	494.3 ± 204.5	483.2 ± 140	884.3 ± 272.2 [#]	878.3 ± 196.7 [#]
Tau	719.7 ± 508.1	639.7 ± 446.8	386.1 ± 207.4 ^{##, #}	224.7 ± 91.1 [#]
Ptau	99.3 ± 52.1	86.6 ± 34.9	65.6 ± 23.9 ^{##, #}	45.3 ± 14.9 [#]
TIV (l)	2.12 ± 0.02	2.18 ± 0.02	2.09 ± 0.03	2.11 ± 0.02 [#]
MTA	1.6 ± 0.8 ^{##}	1.2 ± 0.8	0.6 ± 0.6 ^{##, #}	0.2 ± 0.3 [#]
PA	1.3 ± 0.8	1.3 ± 0.7	0.6 ± 0.7 ^{##, #}	0.3 ± 0.6 [#]
PCA	1 (0.8%)	3 (3%)	-	-
lv-PPA	2 (1.6%)	1 (1%)	-	-

*According to the Verhage system

Values presented as mean ± standard deviation or n (%)

Difference between late-onset AD and old controls; or between early-onset AD and young controls with p < 0.05.

Difference between early- and late-onset AD; or between old and young controls with p < 0.05. Groups were compared using Student's t-tests, Mann-Whitney U-tests and Pearson Chi-Square tests where appropriate.

[§]RAVLT: Dutch version of the Rey Auditory Verbal Learning Test (number of words), TMT: Trail making Test (seconds), TIV: Total Intracranial Volume

Figure 1. The ANCOVA design showed a significant interaction between age and diagnosis, implicating that early-onset AD patients had more pronounced GM reduction in the precuneus than in late-onset AD patients whereas late-onset AD patients had less GM in medial temporal lobe, hippocampus and cerebellum. Brighter colors indicate higher t-values. Figures are displayed with a threshold of $p < 0.001$, uncorrected.

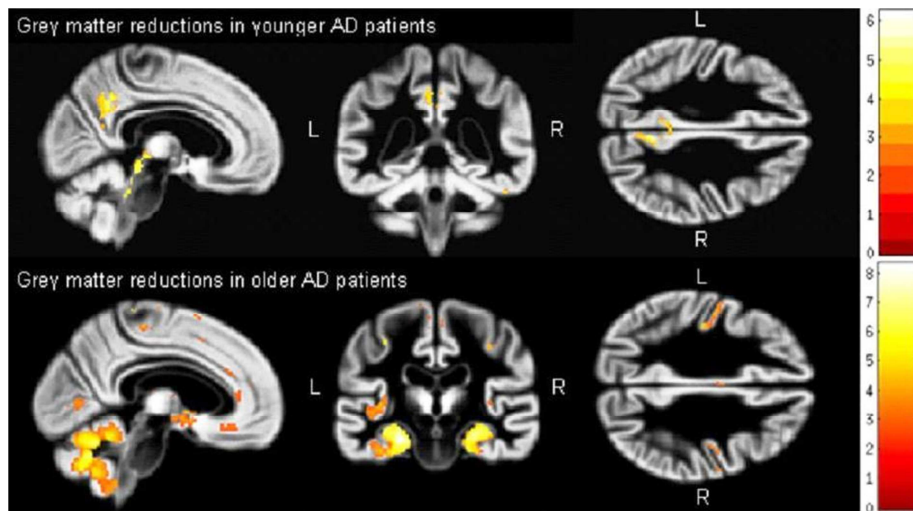


Figure 2. Compared with age-matched controls, late-onset AD patients showed grey matter reductions in hippocampus, left parahippocampal gyrus, left (inferior) parietal lobe, right temporal lobe (middle temporal gyrus, superior temporal gyrus) and cerebellum.

Compared to younger controls, early-onset AD showed widespread reductions in grey matter in hippocampus, parahippocampal gyri, temporal lobes (middle and inferior gyrus), parietal lobes (primarily precuneus and angular gyrus), posterior and anterior cingulate gyrus, and inferior frontal cortex. Brighter colors indicate higher t-values. Figures are displayed with a threshold of $p < 0.001$, uncorrected.

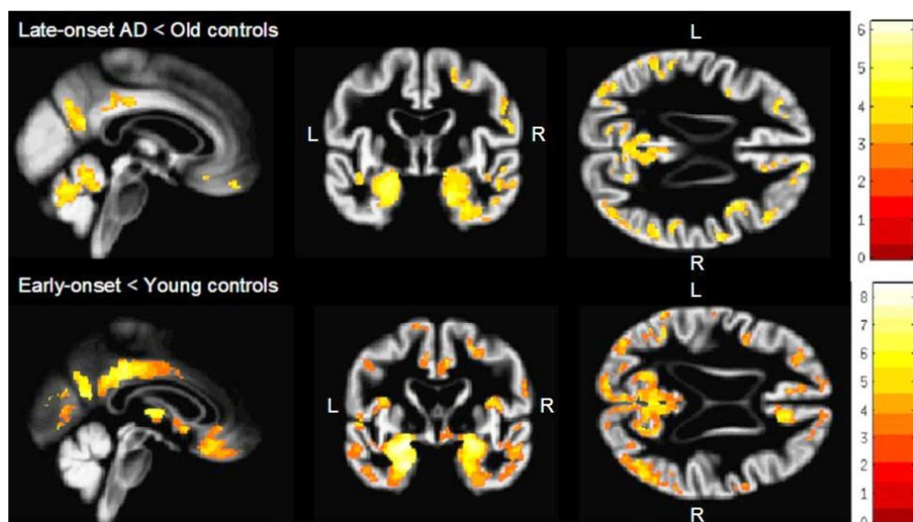


Figure 3. A: Lower MMSE scores in the late-onset AD group were correlated with less grey matter in left temporal lobe. B: Lower MMSE scores in the early-onset AD group were correlated with less grey matter in left and right temporal lobe, but only the first survived the FWE correction. Figures are displayed with a threshold of $p < 0.001$, uncorrected.

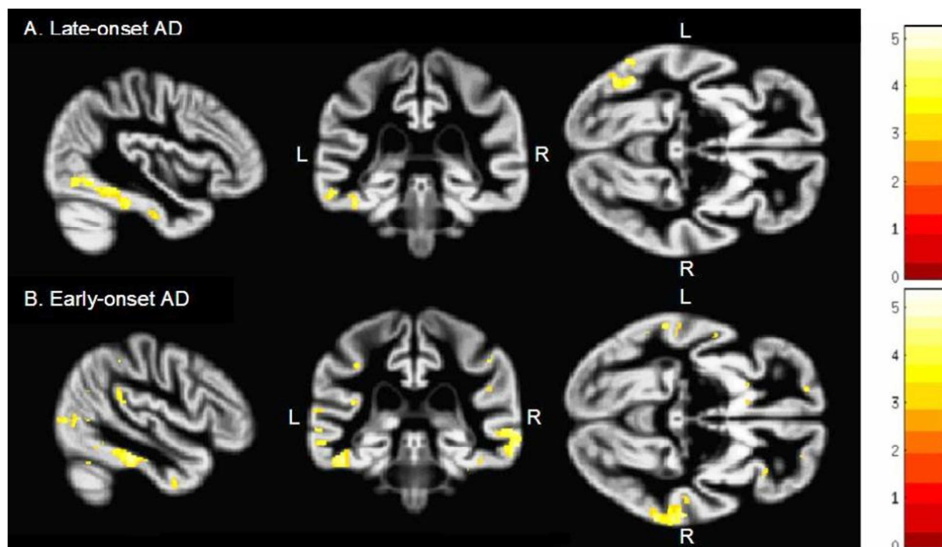
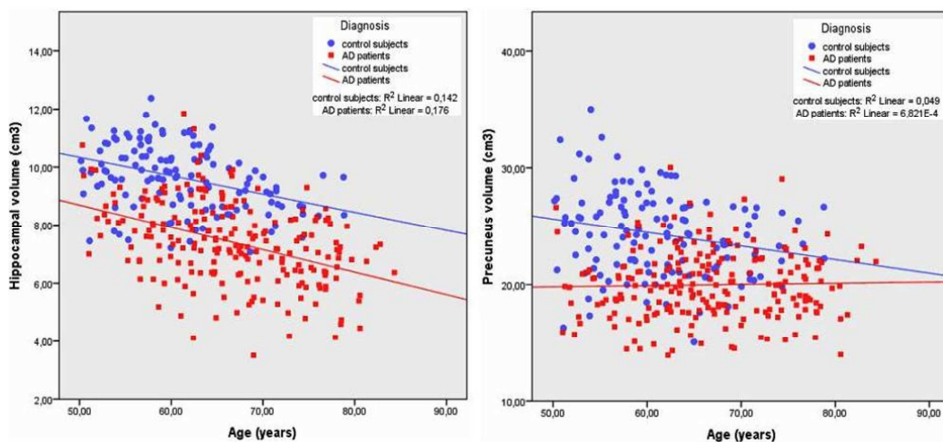


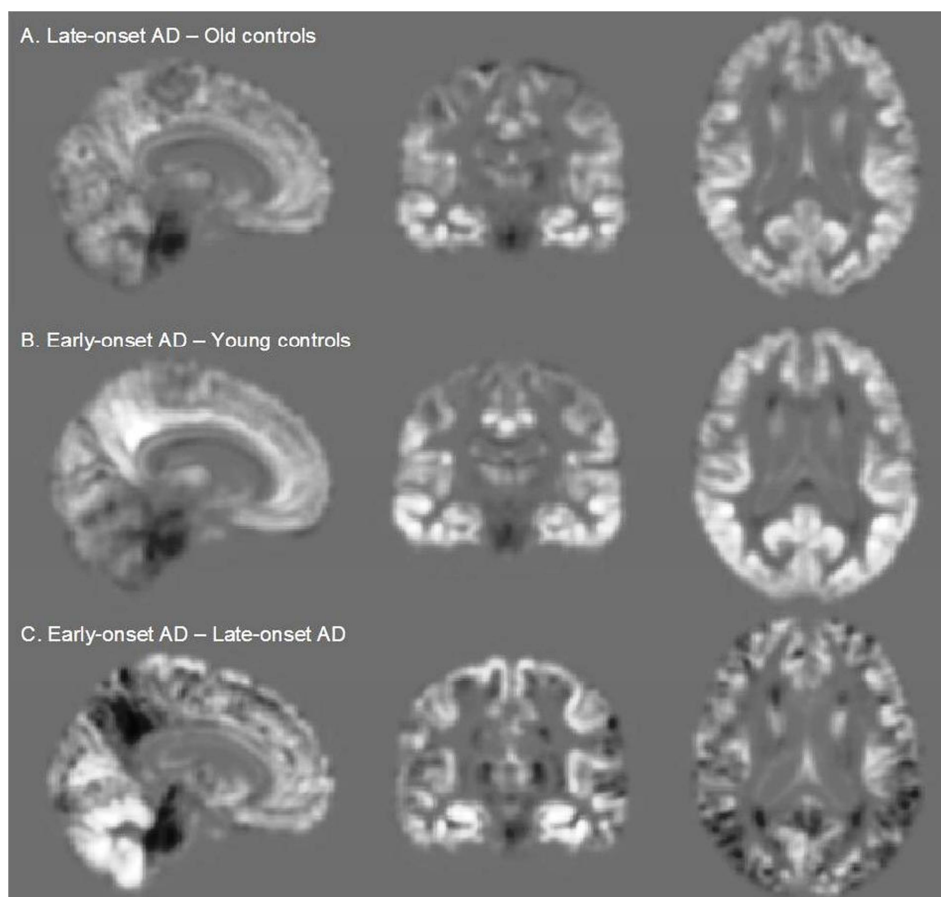
Figure 4. Left: Scatter plot for left and right hippocampus volumes versus age for AD patients (red squares) and controls (blue circles). Lines indicate the regression lines for each group (red for AD, blue for controls). Diagnosis and increasing age independently predicted hippocampal volume. Right: Scatter plot for left and right precuneus volumes versus age for AD patients (red squares) and controls (blue circles). Lines indicate the regression lines for each group (red for AD, blue for controls). The interaction between diagnosis and age showed that precuneus atrophy is most impaired in early-onset AD.



Supplemental material. A: Percent difference maps of group means of late-onset AD patients and old controls. Brighter regions illustrate areas of atrophy in late-onset AD patients. The percent difference map show good agreement with the p-maps of figure 2.

B: Percent difference maps of group means of early-onset AD patients and young controls. Brighter regions illustrate areas of atrophy in early-onset AD patients. The percent difference map show good agreement with the p-maps of figure 2.

C: Percent difference maps of group means of early-onset AD patients and late-onset AD patients. Brighter regions illustrate areas of atrophy in late-onset AD patients, darker regions illustrate areas of atrophy in early-onset AD patients. The percent difference map show good agreement with the p-maps of figure 1.



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